A NOTE ON THE STRUCTURE OF THE LIVING PROTOPLASM OF ECHINODERM EGGS.[1]

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From a study of the living protoplasm of several different echinoderm eggs Wilson[2] came to the conclusion that the structure was alveolar, thus confirming Bütschli. During the past summer in connection with the study of the chemical basis of mitosis I had occasion to repeat his observations and have been led to a somewhat different conclusion.

I examined the living eggs of *Arbacia punctulata* and *Asterias Forbesii* under slight compression, using Leitz $\frac{1}{12}$ oil immersion and ocular 4.

In my opinion the phenomena one observes in the living fertilized egg show that the structure of these eggs is not correctly represented by the term alveolar. The protoplasm seemed to me to consist of a perfectly clear, homogeneous matrix in which no trace of structure could be seen, but which contained a vast number of small granules. These granules differ somewhat in size, those near the periphery of the egg being somewhat larger than at the center, and throughout the egg, but particularly near the surface, a great number of very minute granules or microsomes may be seen.[3]

The description of the protoplasm agrees with that given by Wilson except that he regards these granules as alveoli filled with fluid. That they are more correctly regarded as granules in a homogeneous matrix may be shown in a variety of ways.

For example, the granules may be isolated in sea-water if the egg is first treated with sodium sulphate or sodium iodide, $\frac{5}{8}N$, or even in some cases with neutral red. The granules after such

[1] From the Marine Biological Laboratory, Woods Holl.

[2] Wilson, *Journal of Morphology*, XV., 1899, p. 6.

[3] These observations confirm those of F. R. Lillie on the *Chætopterus* egg (Lillie: Personal communication) and of E. P. Lyon on *Arbacia* (see Lyon: Amer. Jour. of Physiol., 1906, vi).

treatment do not swell and disappear as they ordinarily do on crushing the egg, but persist as granules and may be separated from their surrounding matrix. They then acquire a very active Brownian movement and float off as granules.

That they exist as granules in the egg is shown also by their behavior during mitosis and in the centrifuge. In *Arbacia*, the granules are some of them pigmented. During mitosis, these granules do not remain evenly distributed over the surface of the egg, but collect along the furrow of segmentation. Also when the astral centers are formed the granules either move away from the neighborhood of the asters, as has been described for other eggs, or else they are dissolved in these areas. At any rate near the asters and spindles only the clear protoplasm without granules is to be found.

Furthermore, when the large astral radiations extending to the surface of the egg are formed, it may be seen that these radiations consist of the clear homogeneous matrix. The granules are arranged in rows between the broad clear rays. When oxygen is taken away the granules move in again where the rays were.

Further evidence is obtained if the eggs are centrifugalized in the method discovered by Lyon at Woods Holl, this summer. By centrifugalization the granules are separated into zones of granules, as has been described by Lyon and Lillie and the clear protoplasm forms one zone by itself.

The clear protoplasm which forms the matrix in which the granules are, is oftentimes extremely viscid. This is shown in several ways. The granules possess, as long as the egg remains normal, no Brownian movement, showing that they must be in a viscid matrix. It is only when the egg is partially liquefied that they acquire Brownian movement. The viscidity is also shown by observations on living star-fish eggs partially deprived of oxygen. Long viscid strands can then be seen extending between the blastomeres, with here and there granules embedded in them.[1] If these eggs are beaten with a rod, it is frequently possible to draw the protoplasm out in long clear threads.

[1] See Mrs. Andrews: Journal of Morphology, 1896, xii, p. 367.

I do not mean to imply that these granules are hard insoluble granules because many of them, as Wilson has pointed out, appear more fluid than solid, but I mean that the actual structure of protoplasm is more clearly and accurately represented as a clear homogeneous viscid matrix, in which oil globules and all sorts and sizes of granules are embedded than as an alveolar structure.[1] An alveolus is a hole in a matrix. It is not the hole, but the matrix, which in my opinion is the important part of protoplasm.

If one seeks for a substance most nearly resembling protoplasm in structure, raspberry jam, were it colorless, would fairly accurately represent what one sees — a clear sticky matrix in which are a multitude of seeds.

In none of the eggs while living could I see any trace of spindle fibers or of fibers at all like those seen in sections. When the egg is fixed, the proteid colloids which in solution constitute the larger part of the clear viscid matrix, are precipitated in a granular form. In other words, the clear matrix appears to represent a colloidal solution in which our fixing agents throw down a precipitate. The appearance of rays seen in fixed preparations is quite different from appearances in the living egg. In the living egg the clear protoplasm sends off radiating broad strands toward the periphery of the cell. In fixed preparations the rays appear a great deal stiffer and much finer, not so broad as the clear bands although they occupy the position of the living radiations.

These observations indicate very clearly to my mind that the protoplasm of these eggs is essentially a viscid colloidal solution something like gum arabic in viscidity and embedded in it are precipitated or undissolved granules. It may be regarded as a partially precipitated, colloidal solution and the ease with which the granules may be made to dissolve in the clear matrix, indicates, I think, that the amount of precipitate and number of these granules must be constantly changing. Protoplasm appears to be in fact a two-phase colloidal system, undergoing spontaneous chemical change, and surrounded by a semi-permeable membrane.

The changes the granules undergo in the living egg when the latter is compressed or burst are extremely difficult to follow and

[1] From *Asterias* eggs large quantities of an oil may be separated.

interpret. The granules fade out or seem to dissolve in whole or in part in the viscid matrix. This matrix with the dissolved granules, when in contact with water, rounds itself up as if oily and the crushed protoplasm at first as it flows from the egg breaks into a mass of spheres consisting of viscid matter with dissolved granules, and these spheres then ultimately dissolve almost completely in the water.

The speed of solution of the granules is extraordinary. In some cases where the egg was too much compressed by the coverglass and lens, in a few seconds, almost all the granules in the egg disappeared.

These granules all stain in the basic dyes *intra vitam*, and never in acid. I found, however, that if eggs which have died in the ovary are brought into the dyes, all the dead eggs stain in the acid dyes; all the living in the basic. A change in staining reaction, probably coincident with a change in reaction from alkaline to acid, thus occurs at death.

If eggs in which the granules are stained with any basic dye be crushed, it will be observed that the color disappears when the granule dissolves. What becomes of the color is a puzzle. It does not stain the matrix nor appear to color the sea-water. It may be that it is destroyed by the chemical change taking place.

The extreme sensitiveness of the granules, and the abruptness with which the change in appearance of the protoplasm occurs, suggests that possibly this is a physiological process to be correlated with some of the vital phenomena, *i. e.*, contractility.

Dr. R. G. Davis examined, at my suggestion, the chemical composition of the star-fish egg. He succeeded in isolating from the eggs a large amount of an oil, fluid at ordinary temperatures. The chemical nature of this oil has not been determined. I think it possible that many of these granules represent that oil. The oil is very likely partially saponified and contains a small amount of fatty acid. By the dissociation of this acid the drop or oil globule becomes electro-negative. It is in this way the globule acquires the power of staining with basic dyes, since the insoluble soap is formed. The quick disappearance of the granules when compressed, giving one the impression that they are composed of a fluid which mixes with the viscid matrix, and

their quick fusion under the influence of ether, also bear out this interpretation. It is not impossible that ordinarily the granules or oil drops are in combination of a peculiar kind with basic proteids of the protoplasm, the whole forming a highly unstable and complex compound. The chemical composition of these granules is being studied more closely.